

Figure 1B: siNA Hybridization Assay

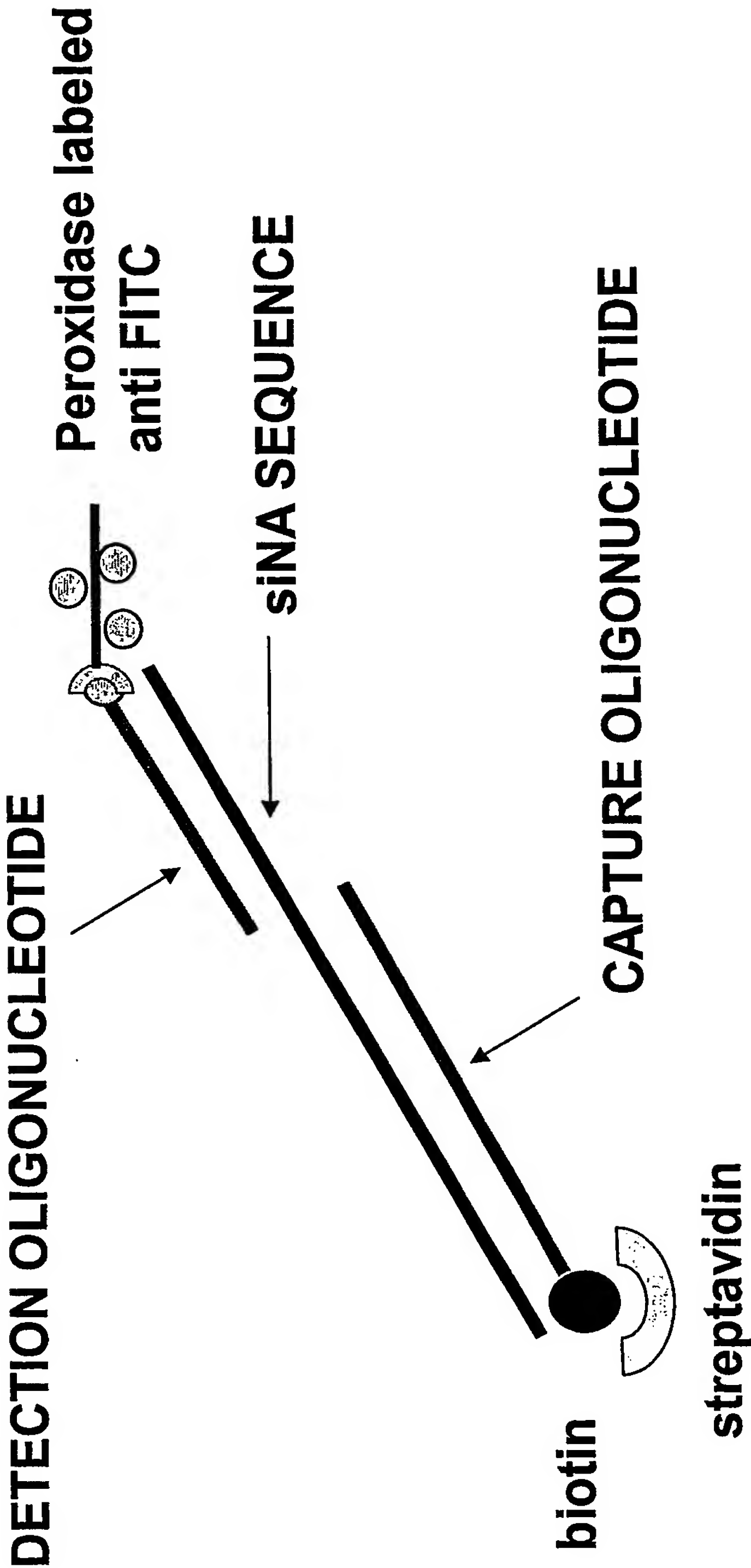
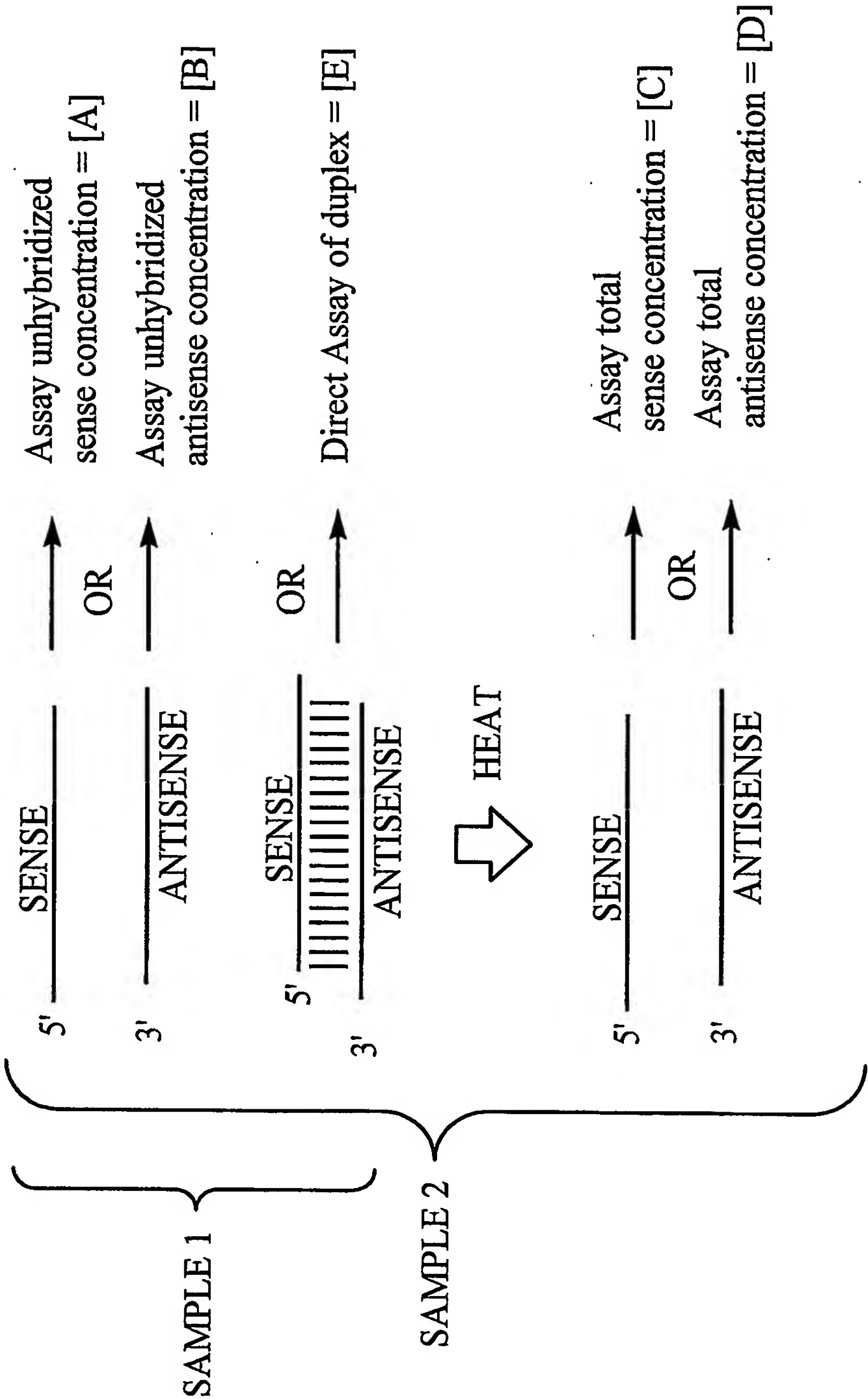
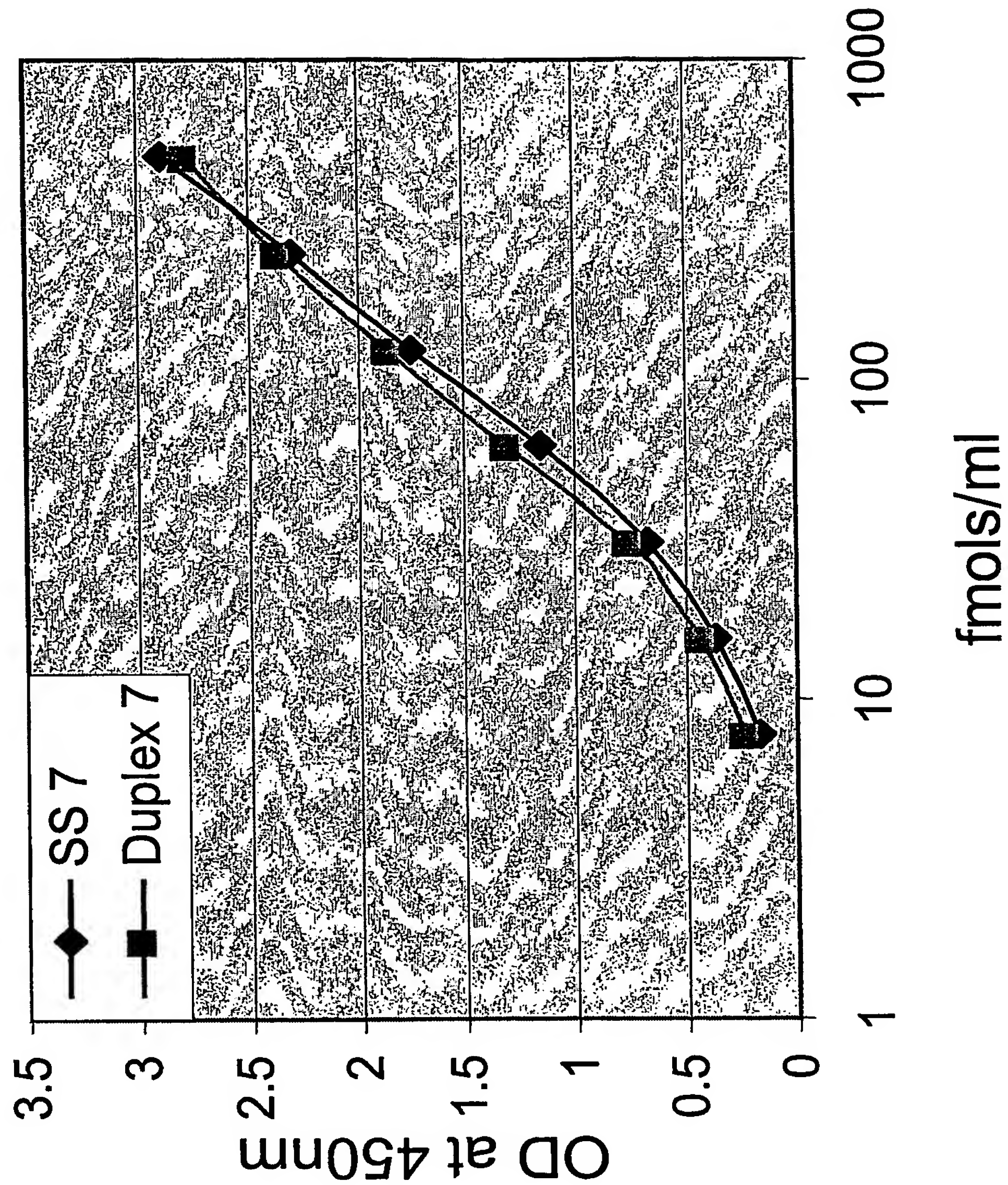


Figure 1C: Principle of siNA detection/quantitation Assays



$[C] - [A] = [\text{Duplex}]$ based upon analysis of sense strand
 $[D] - [B] = [\text{Duplex}]$ based upon analysis of antisense strand
 $[E] = [\text{Duplex}]$ based upon direct analysis of duplex

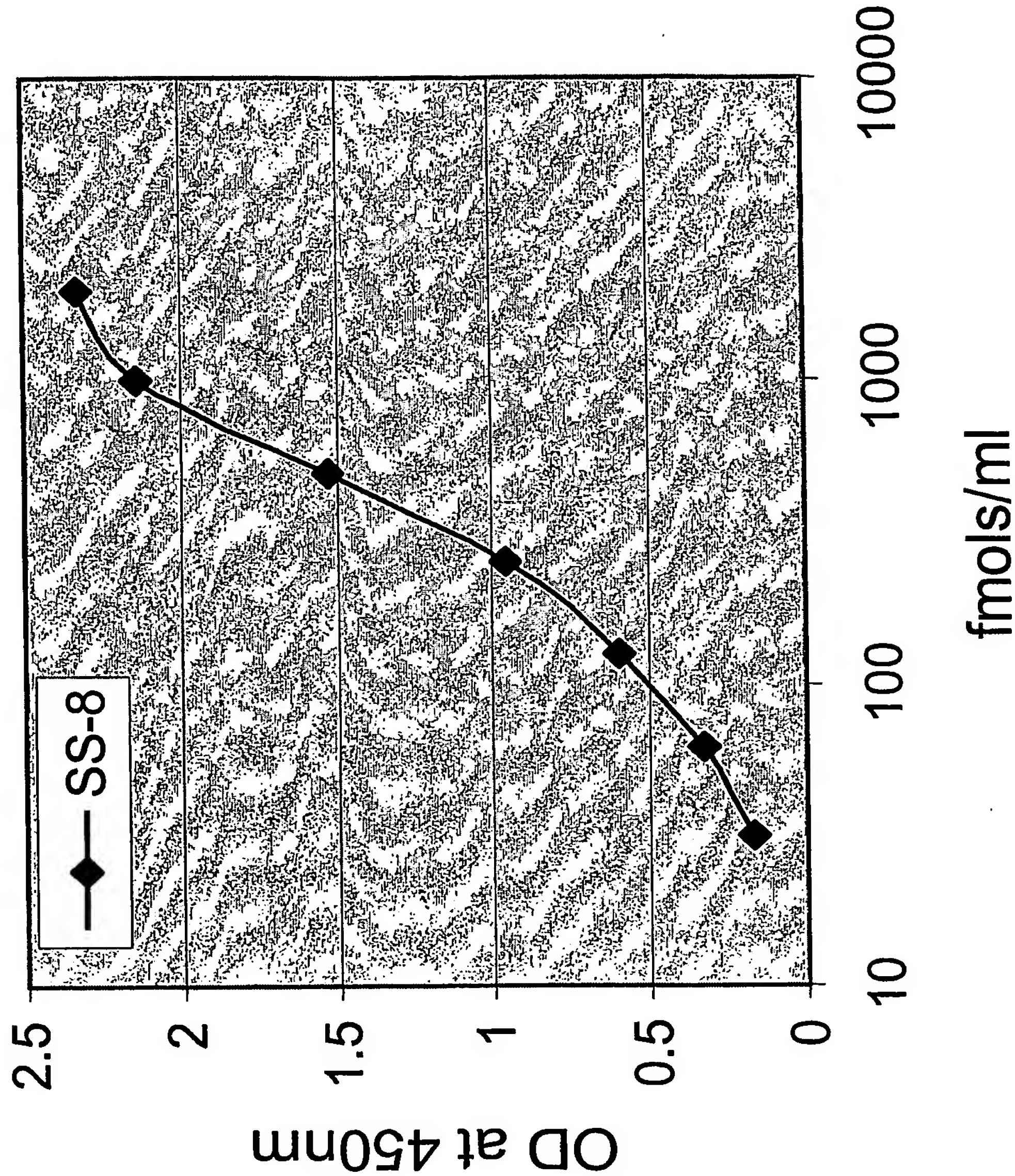
Figure 2A: siNA Stab 7 Sense Strand Standard Curve



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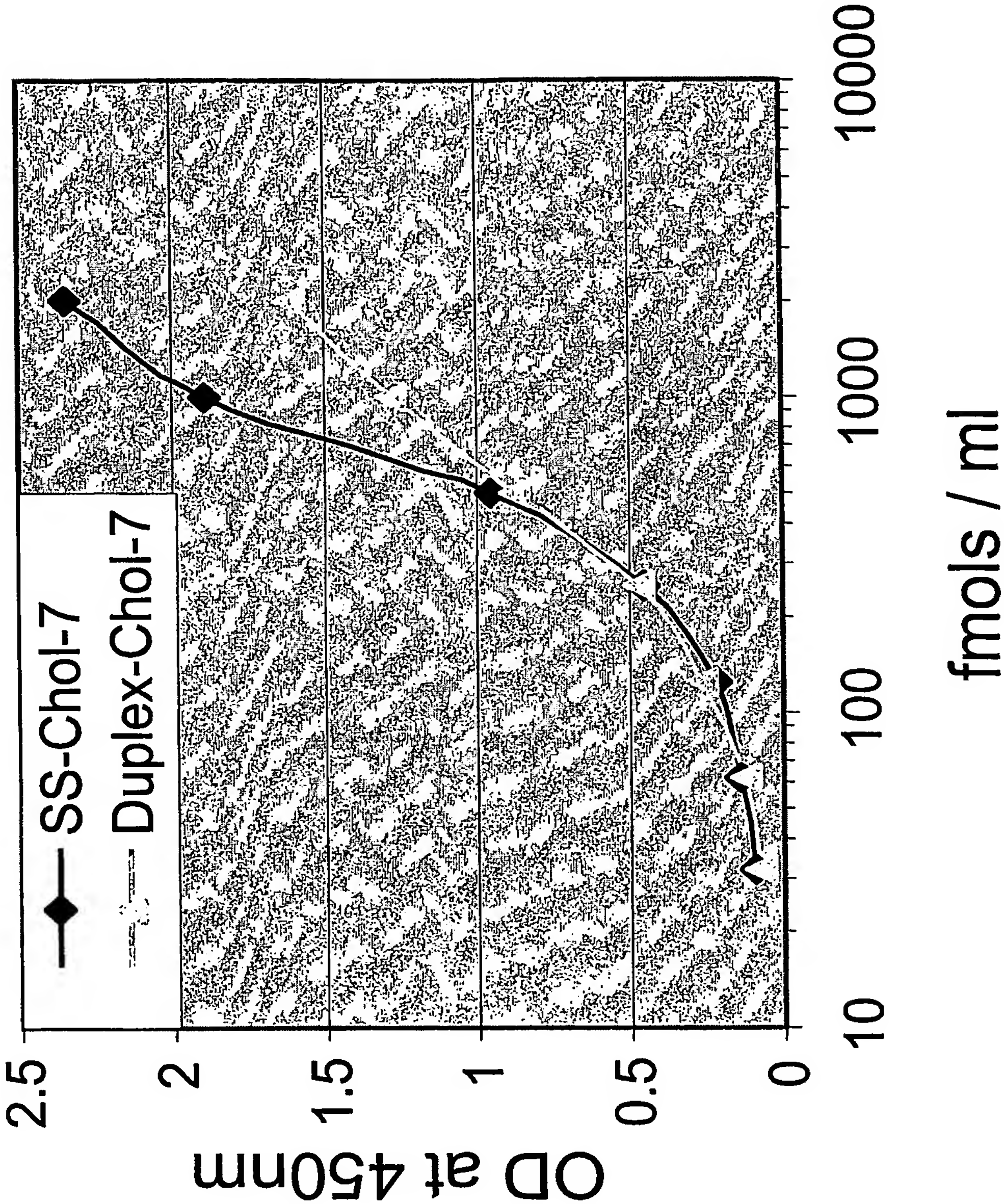
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Figure 2B: siNA Stab 8 Antisense Strand Standard Curve



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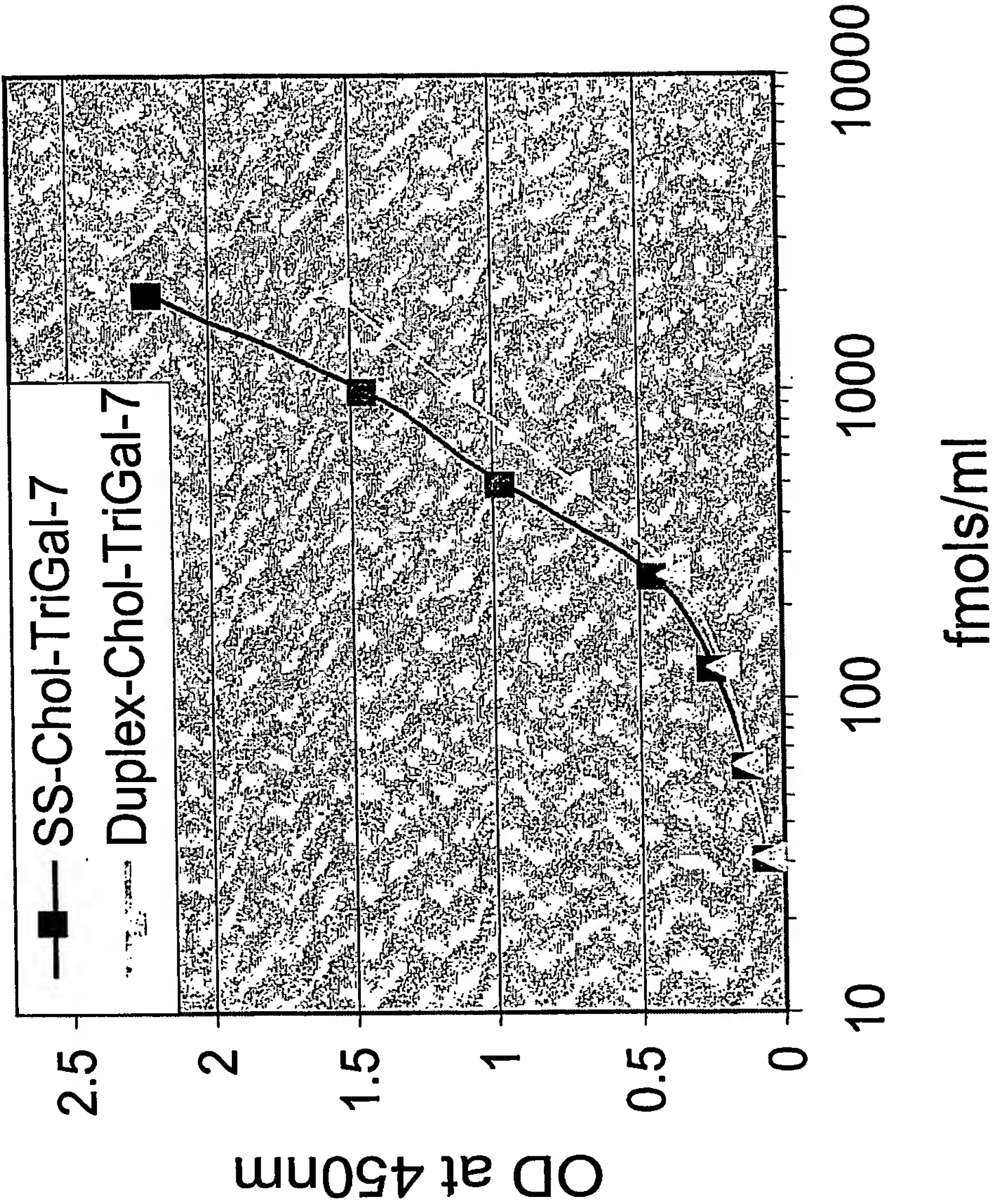
**Figure 2C: siNA Stab 7 Cholesterol Conjugate
Sense Strand Standard Curve**



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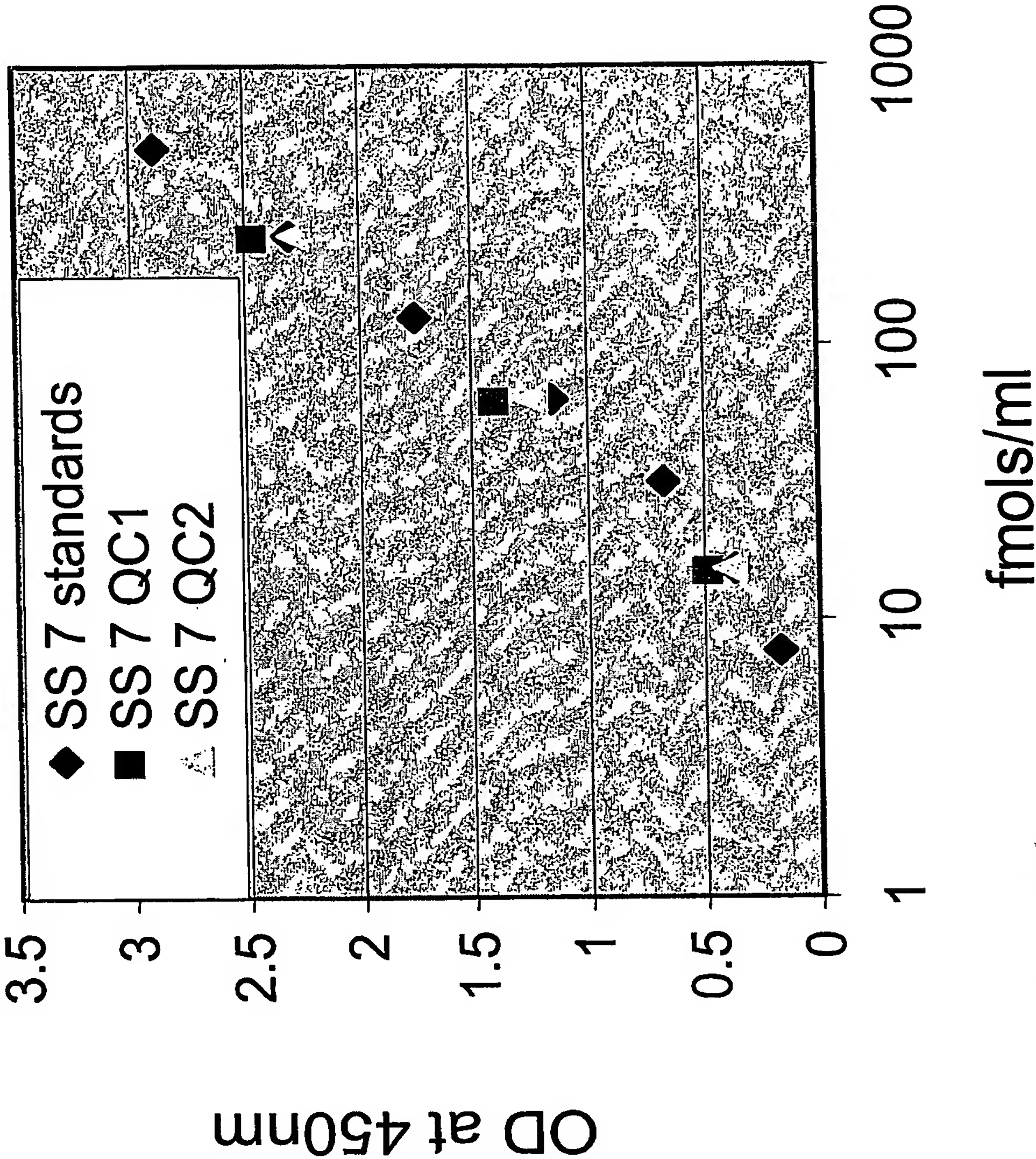
**Figure 2D: siNA Stab 7 Trigalactose Cholesterol
Conjugate Antisense Strand Standard Curve**



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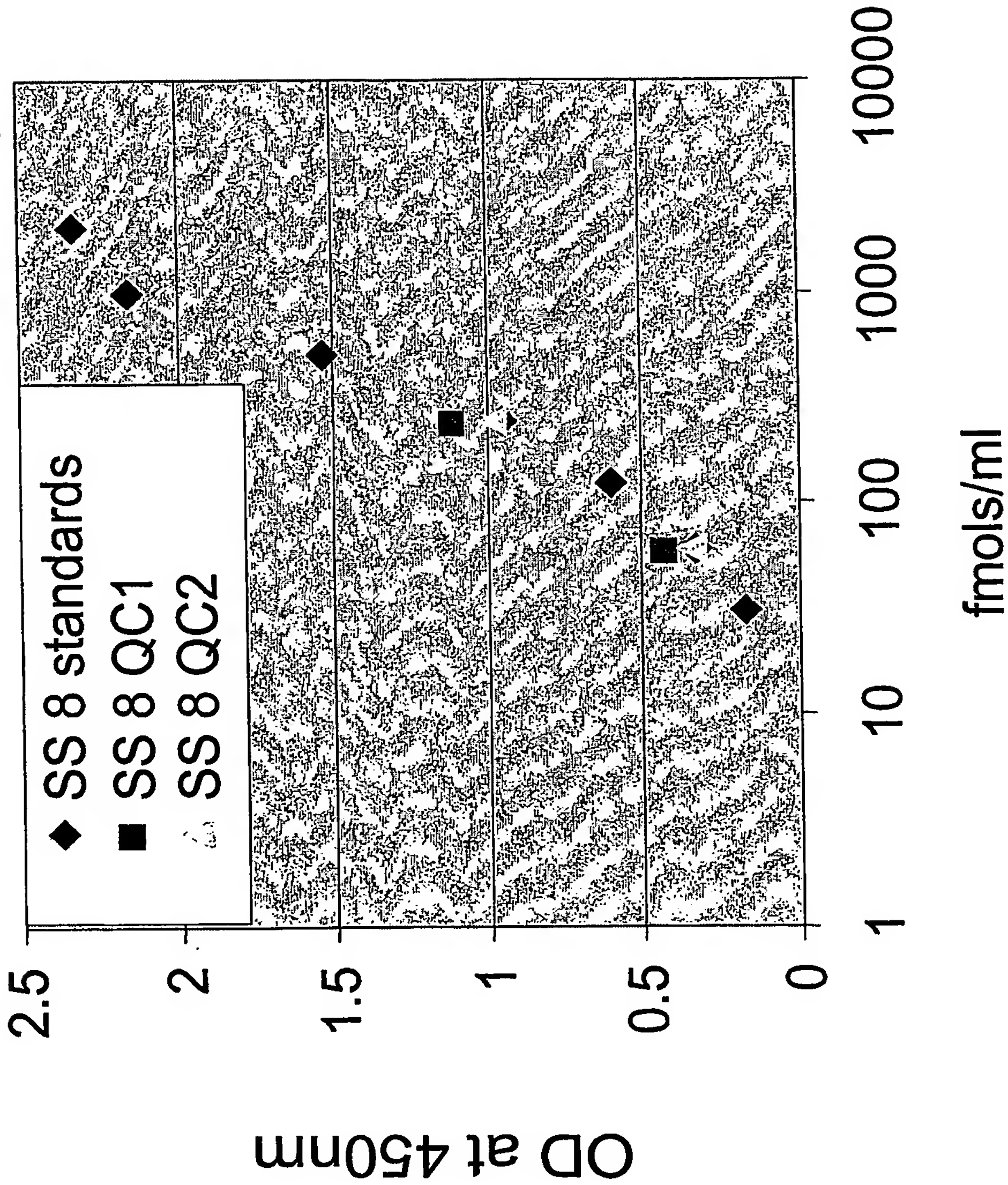
Figure 3A: siNA Stab 7 Single Stranded
Quality Control Sample



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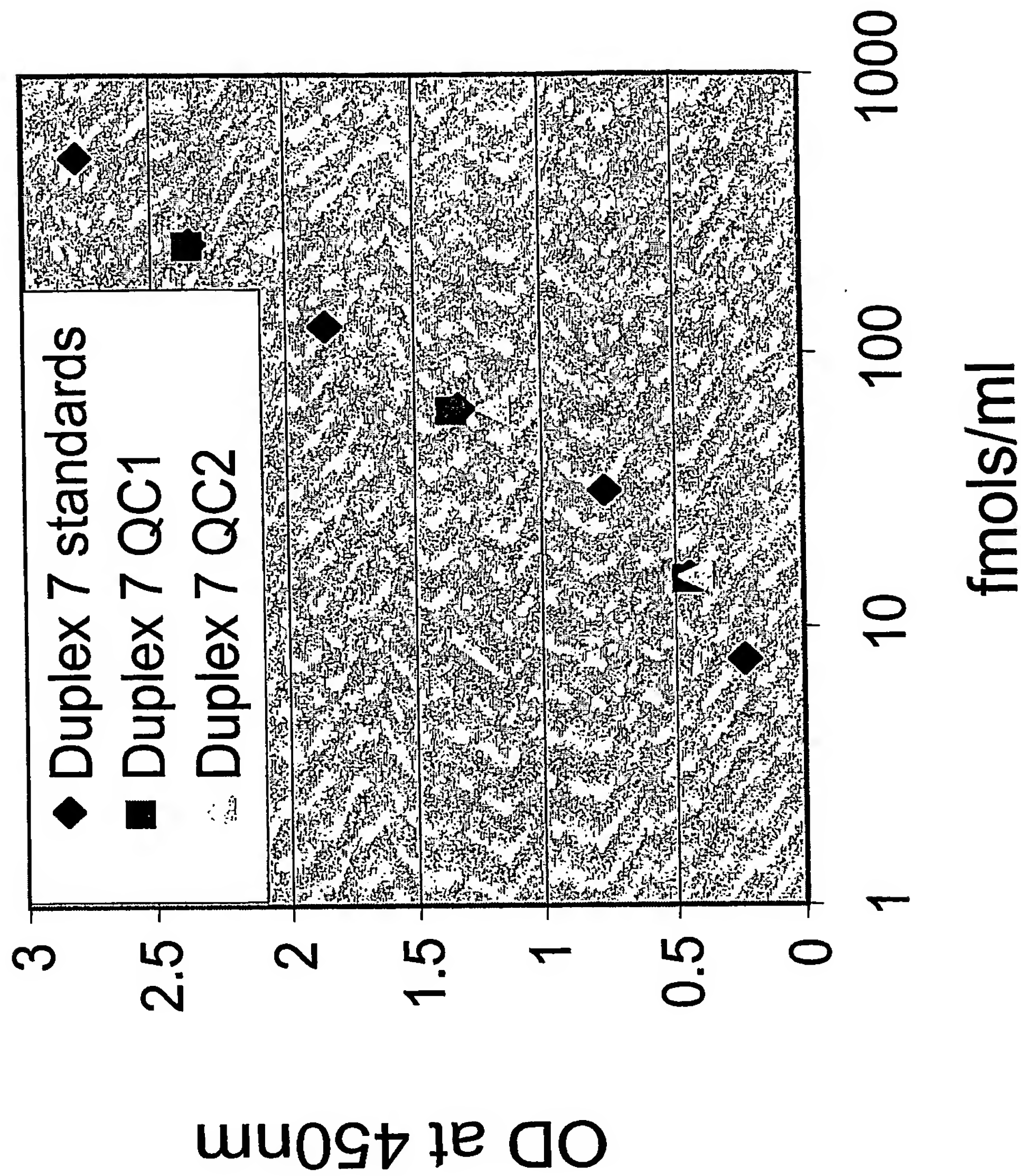
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**Figure 3B: siNA Stab 8 Single Stranded
Quality Control Sample**



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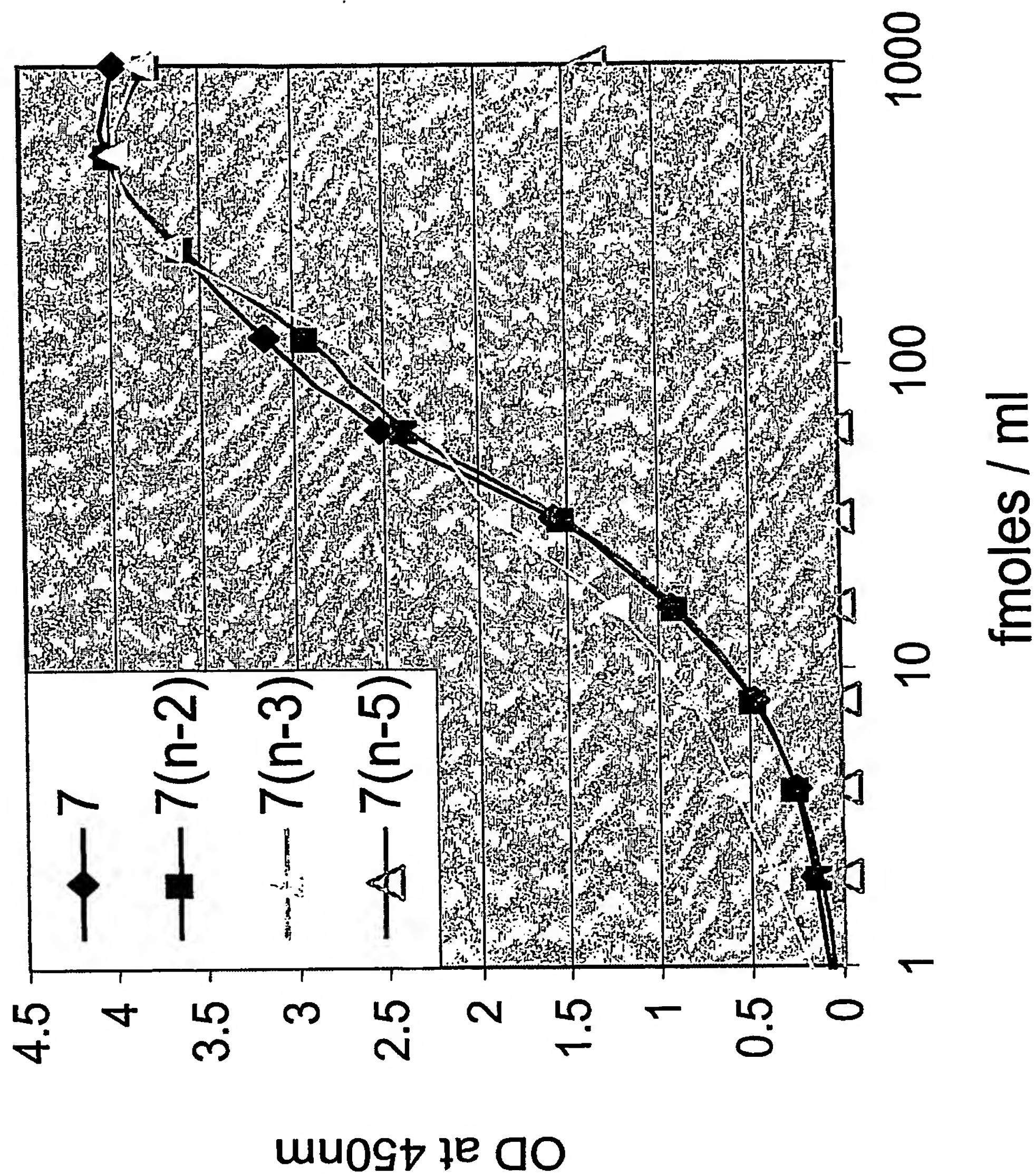
**Figure 3C: siNA Stab 7 Duplex
Quality Control Sample**



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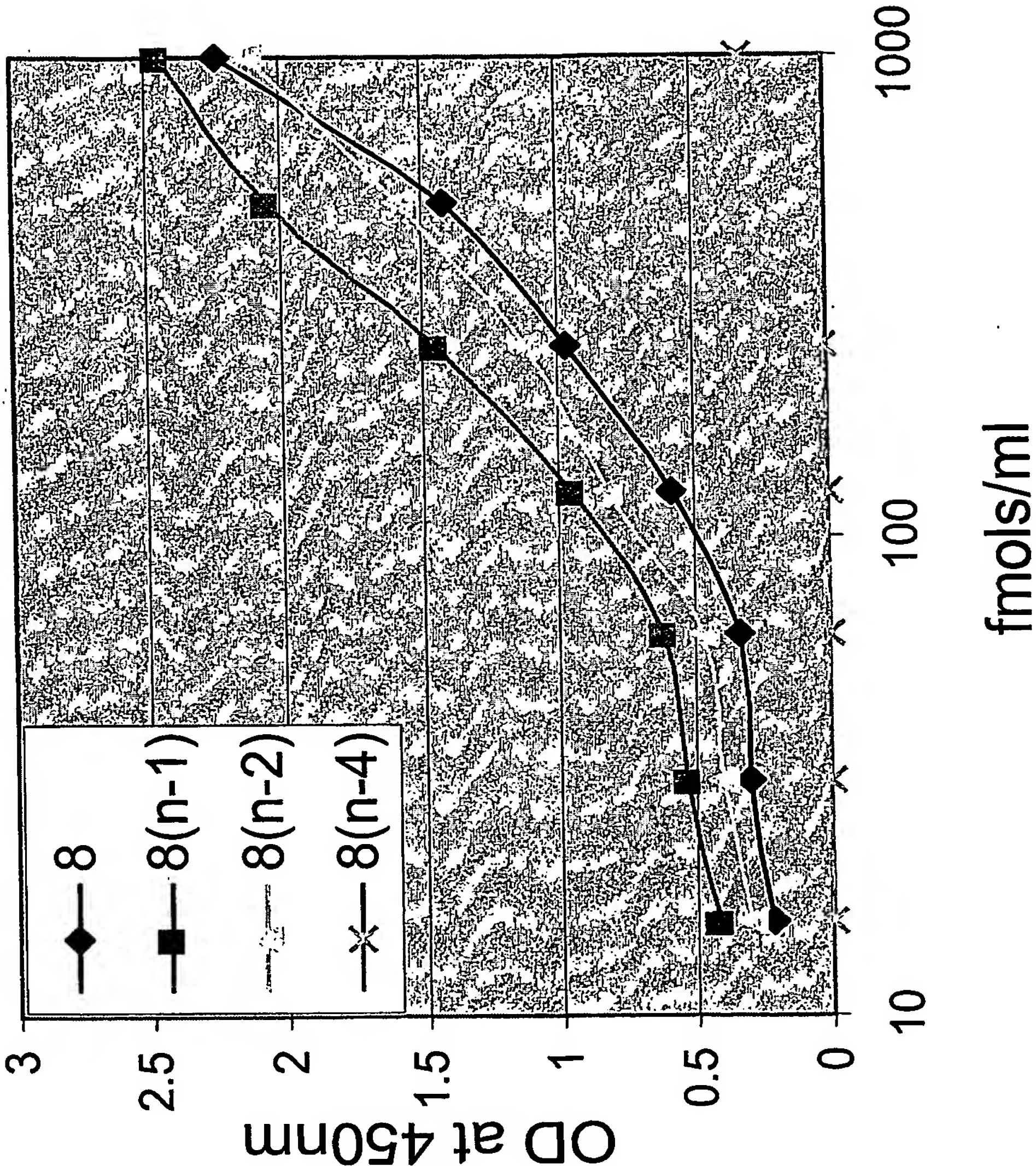
**Figure 4A: Detection of potential
siNA Stab 7 Metabolites via Hybridization Assay**



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**Figure 4B: Detection of potential
siNA Stab 8 Metabolites via Hybridization Assay**



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Figure 5A: Effect of Hepatocyte lysate on detection of single stranded Stab 7 cholesterol conjugate siNA sequence

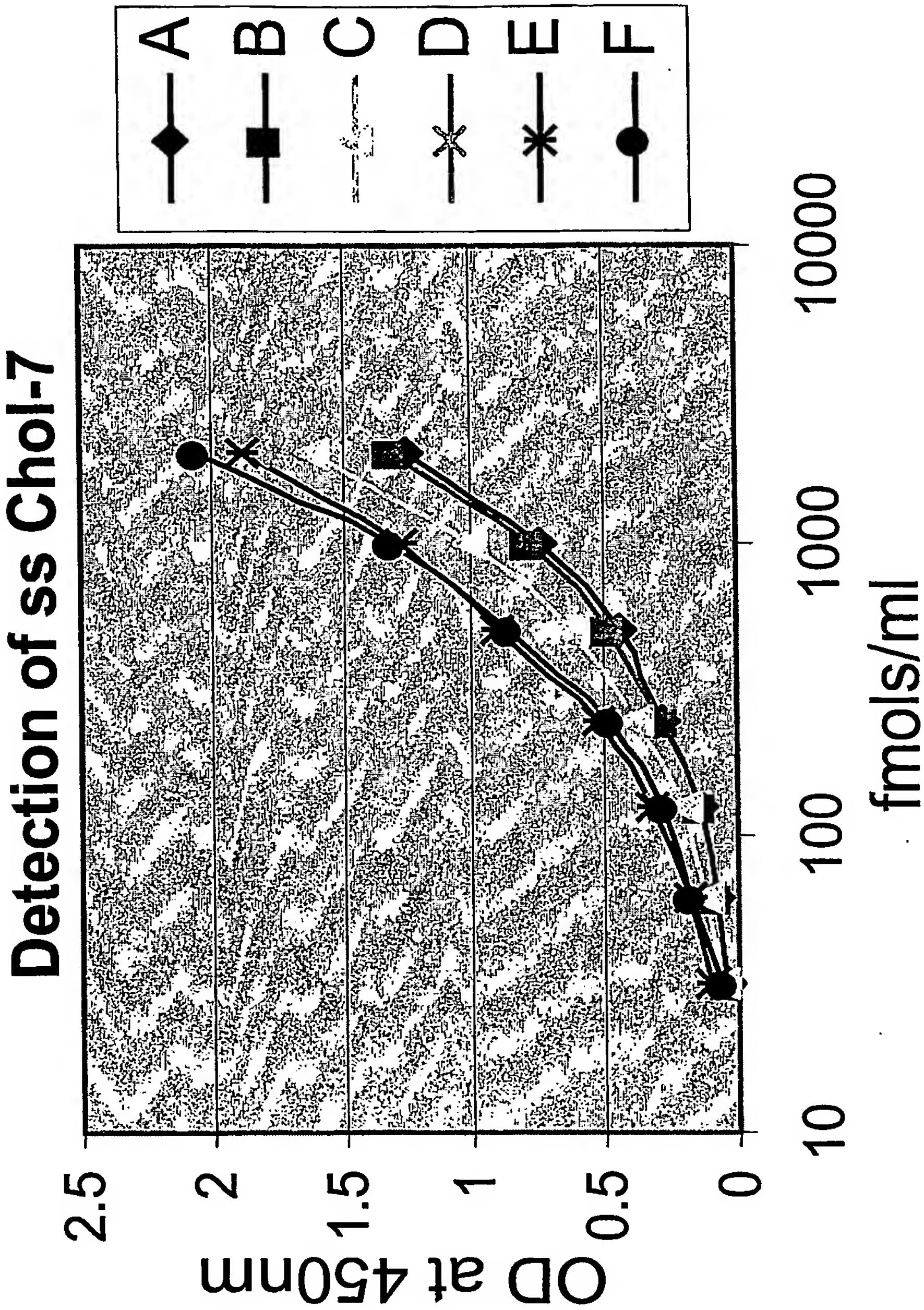
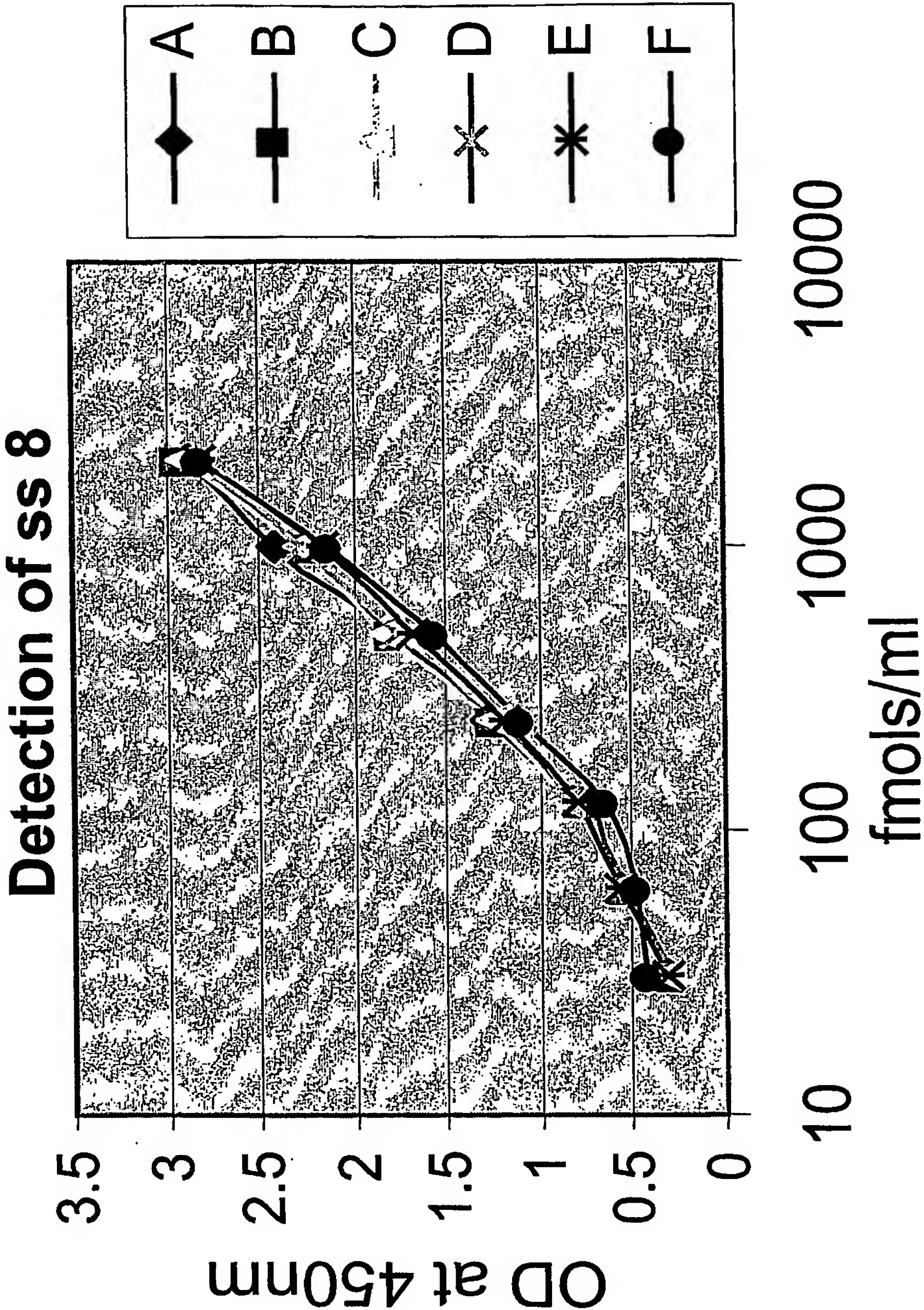
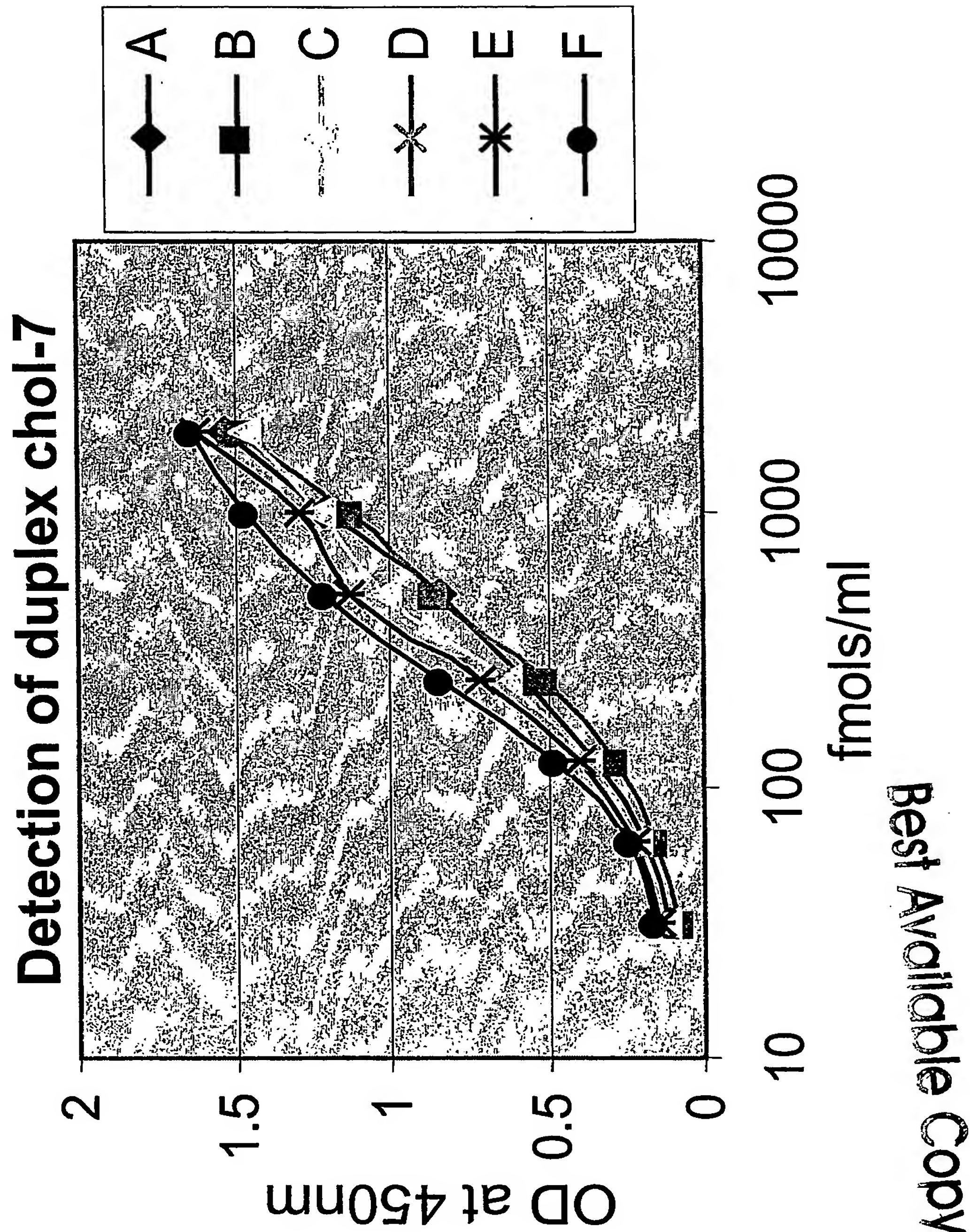


Figure 5B: Effect of Hepatocyte lysate on detection of single stranded Stab 8 siNA sequence



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Figure 5C: Effect of Hepatocyte lysate on detection of Stab 7 cholesterol conjugate duplex siNA sequence



**Figure 6A: Effect of monkey plasma
on detection of single stranded Stab 7 cholesterol
conjugate siNA sequence**

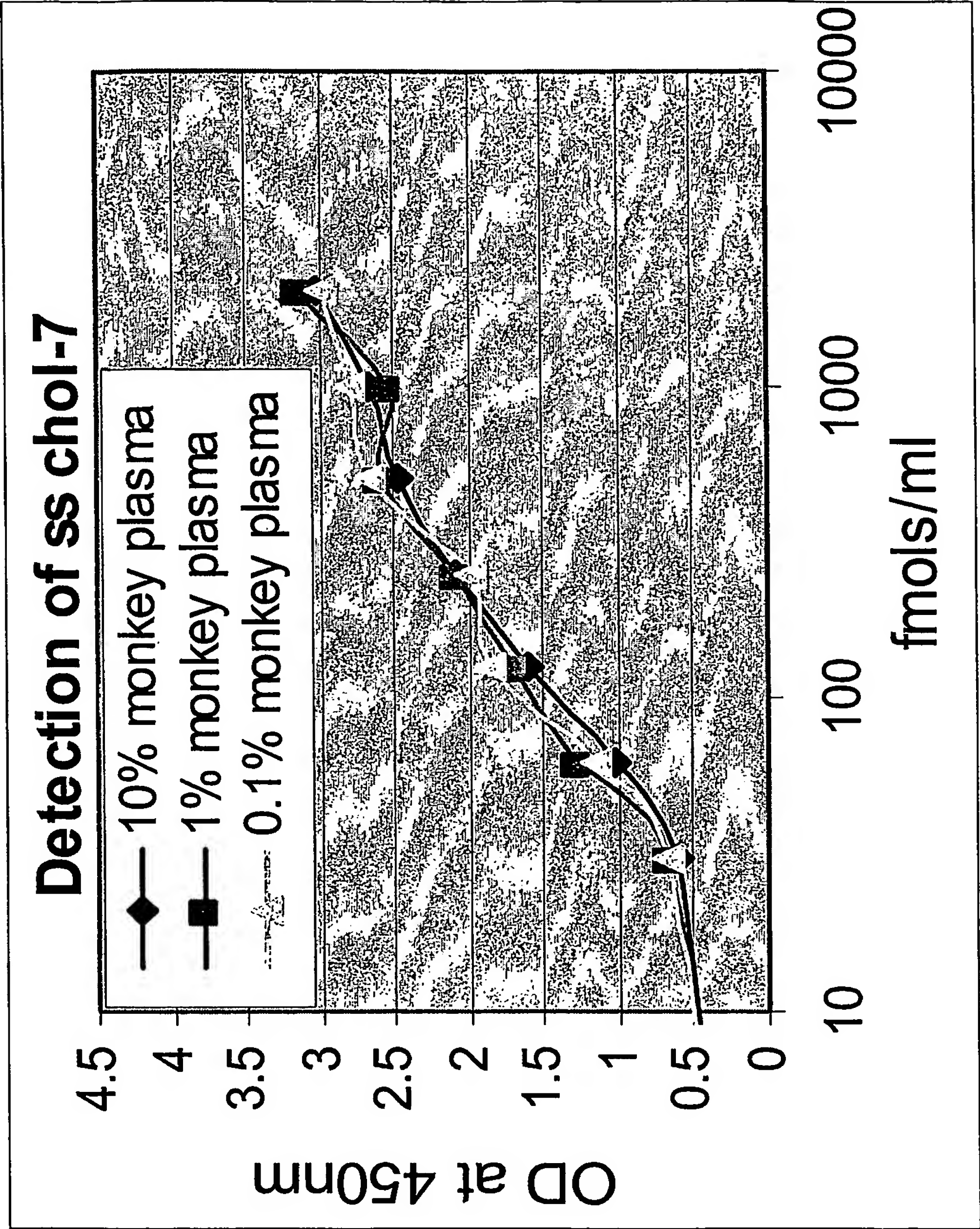


Figure 6B: Effect of monkey plasma on detection of single stranded Stab 8 siNA sequence

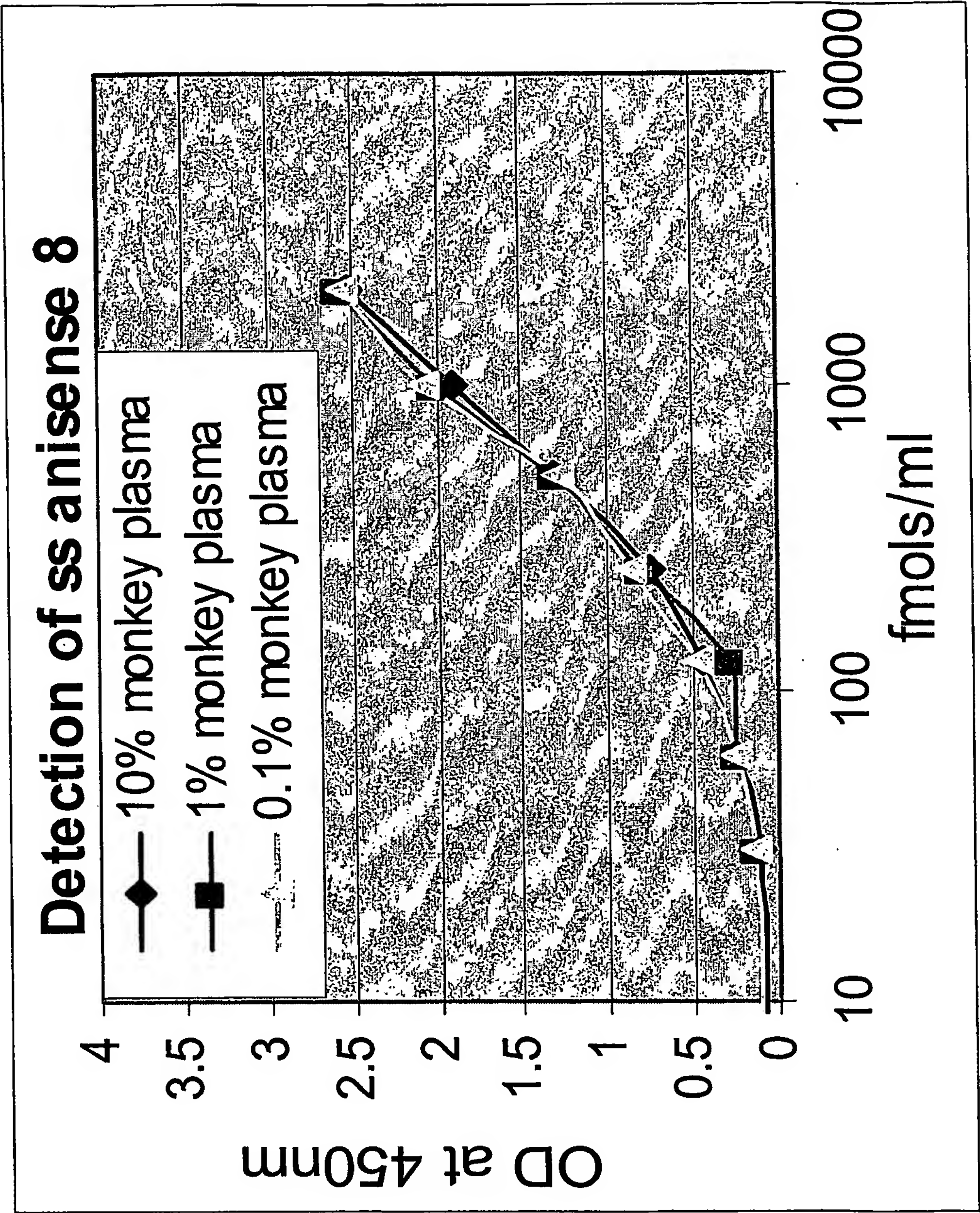
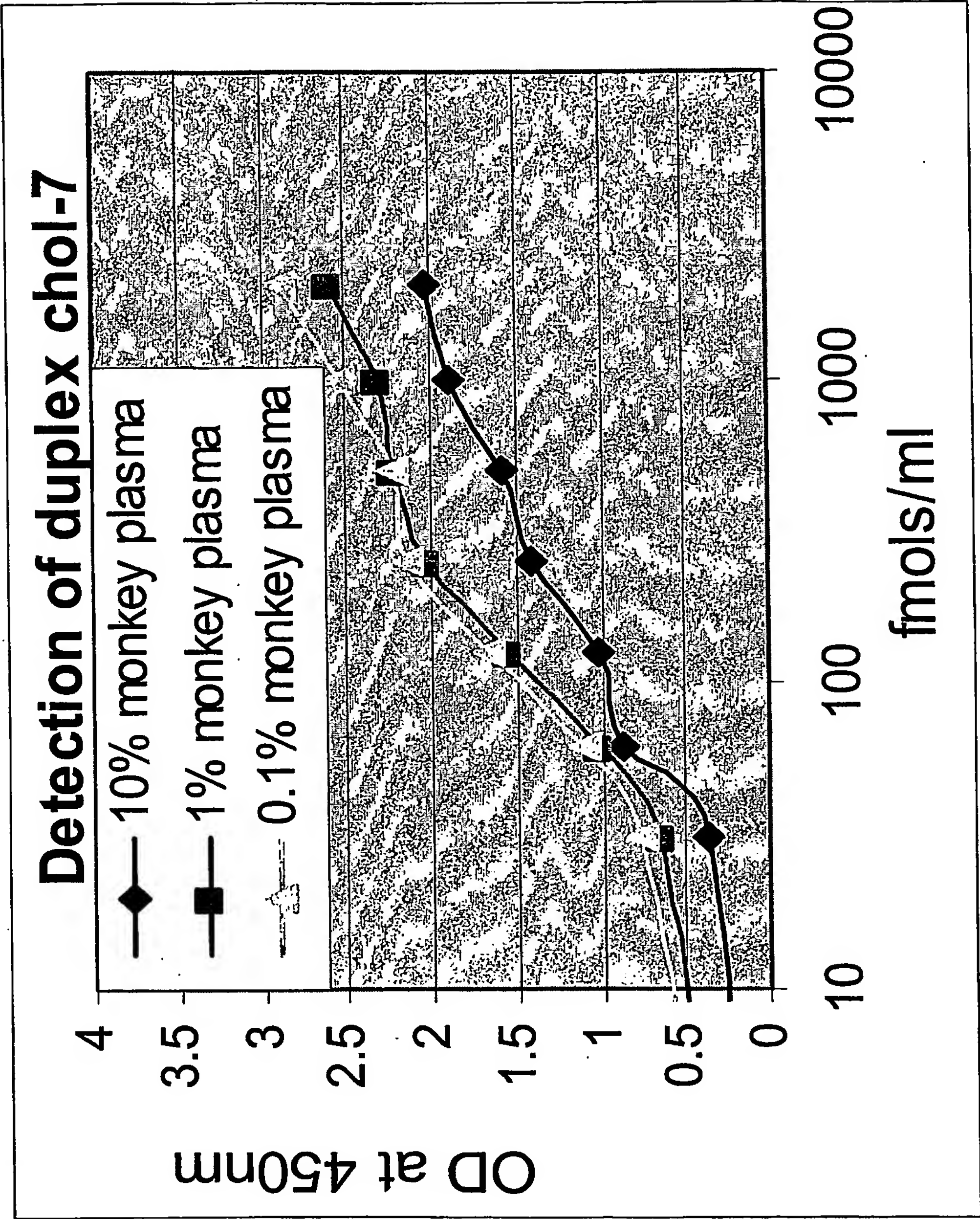
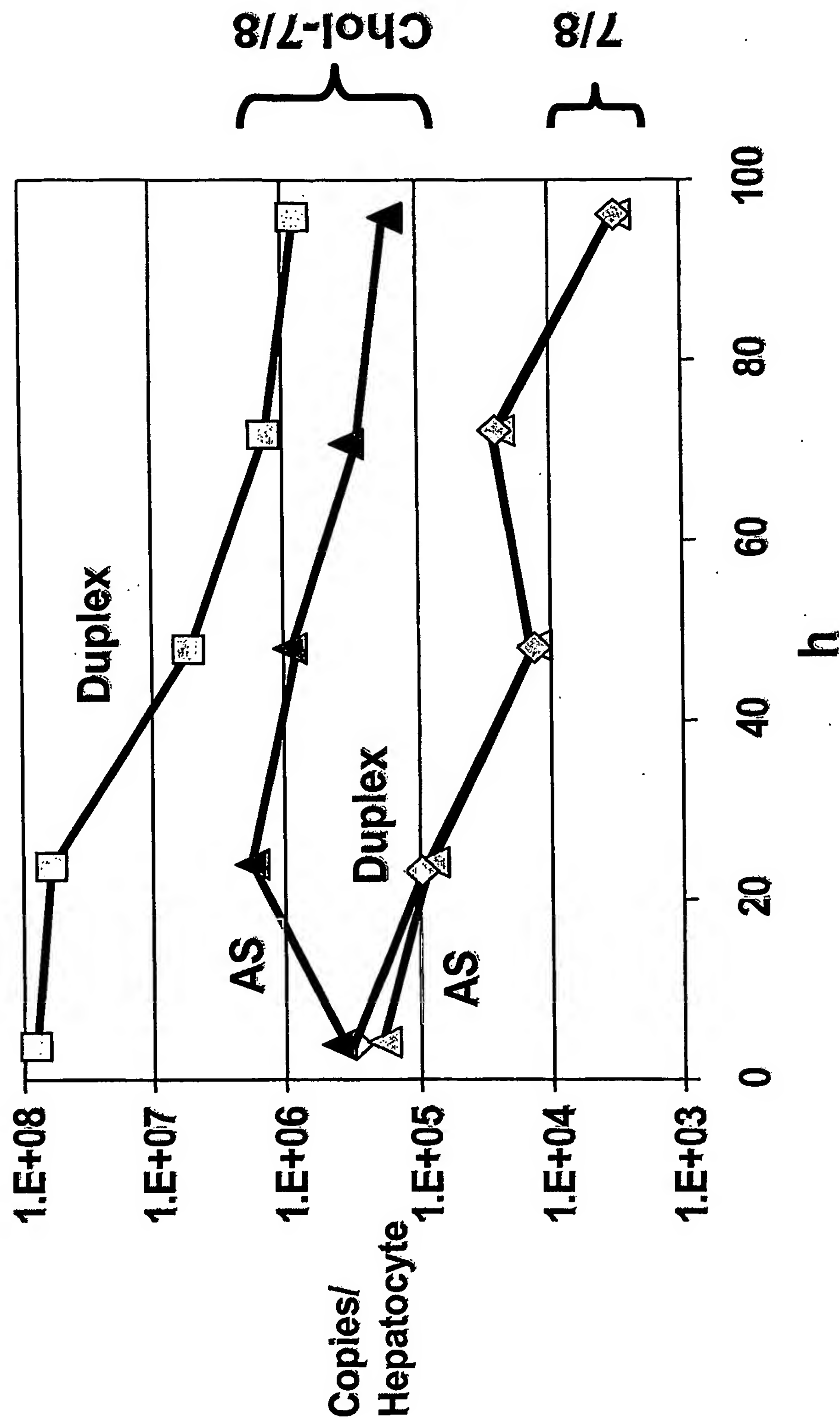


Figure 6C: Effect of monkey plasma on detection of Stab 7 cholesterol conjugate duplex siNA sequence



**Figure 7: Concentration of siNA duplex and antisense
In Hepatocytes**



Given - 1 gram liver = 1 mL = 10¹⁰ cells [siRNA ~ uM]

Figure 8: Removal of Competitive binding sequence
In duplex assay

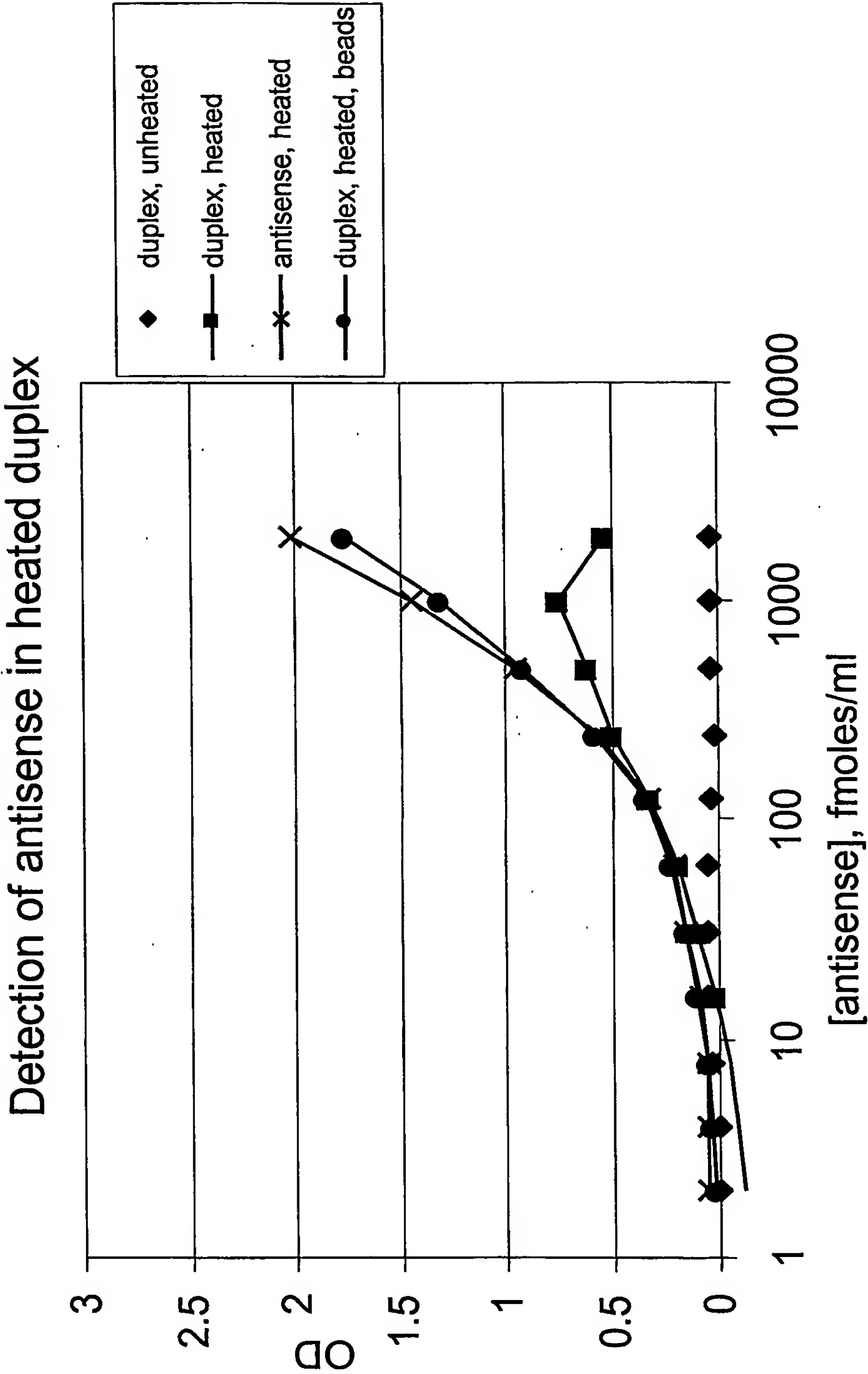
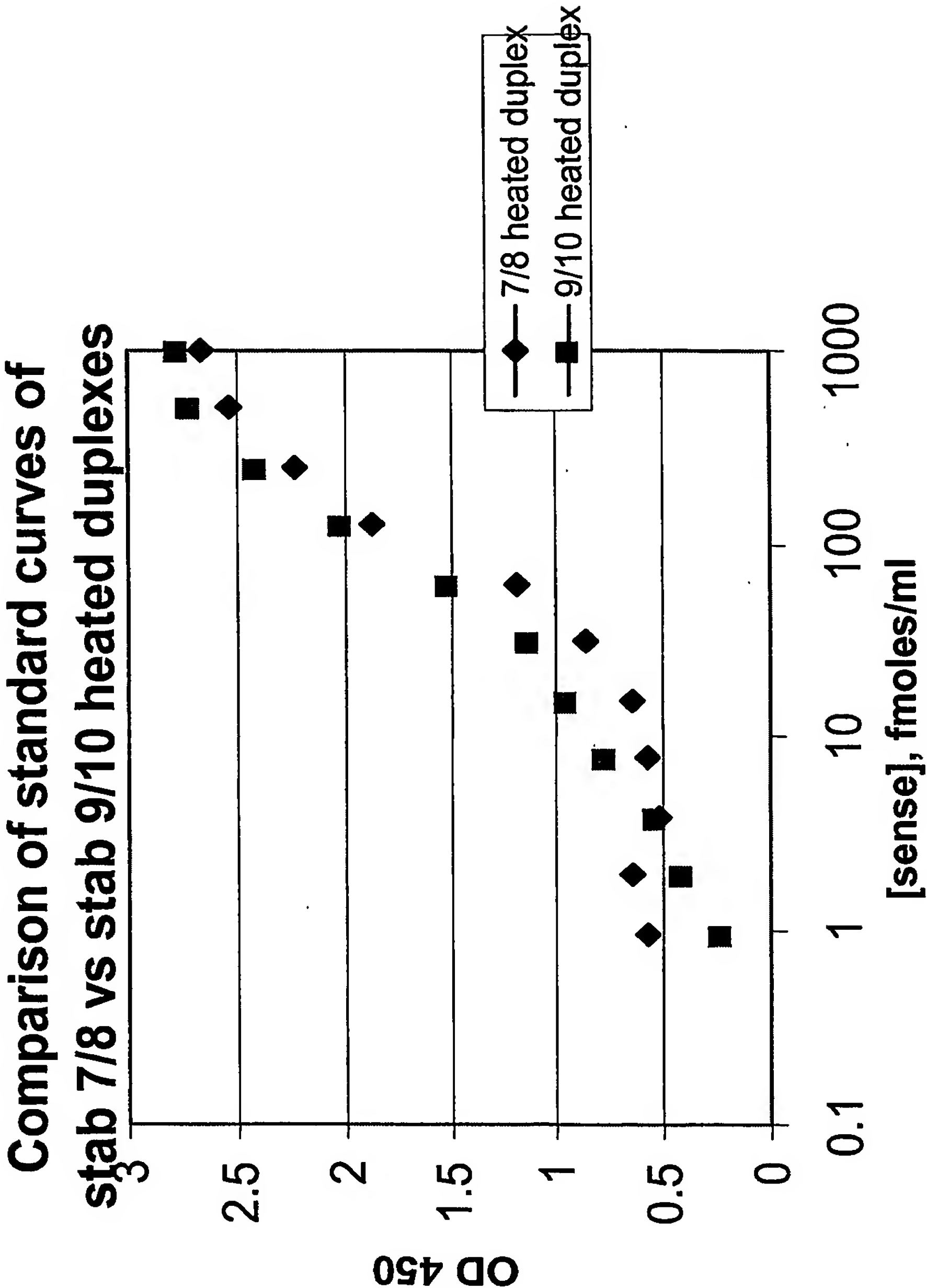
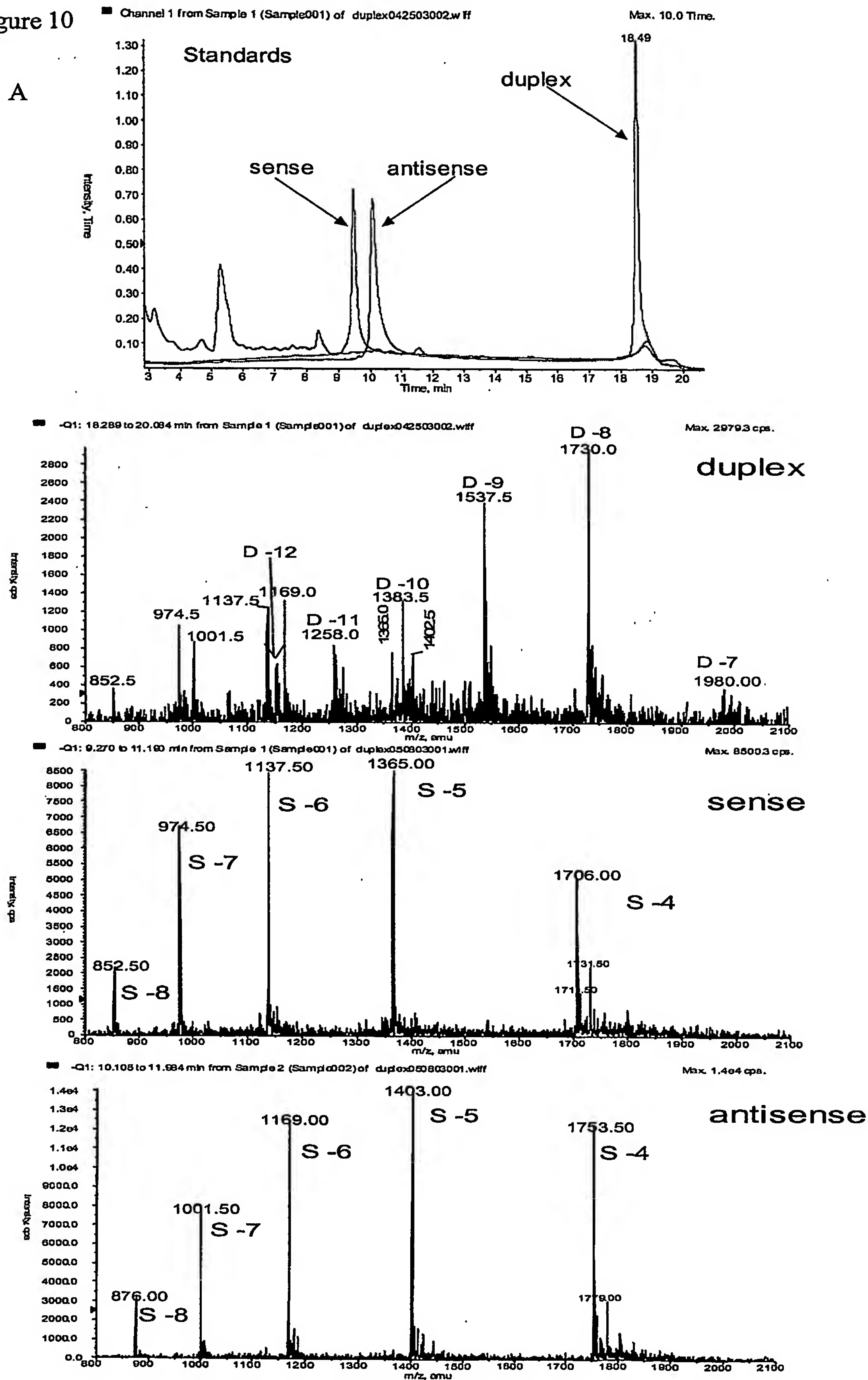


Figure 9: Application of Hybridization Assay to siNA molecules having identical sequence with differing chemical modifications



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Figure 10



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Figure 11

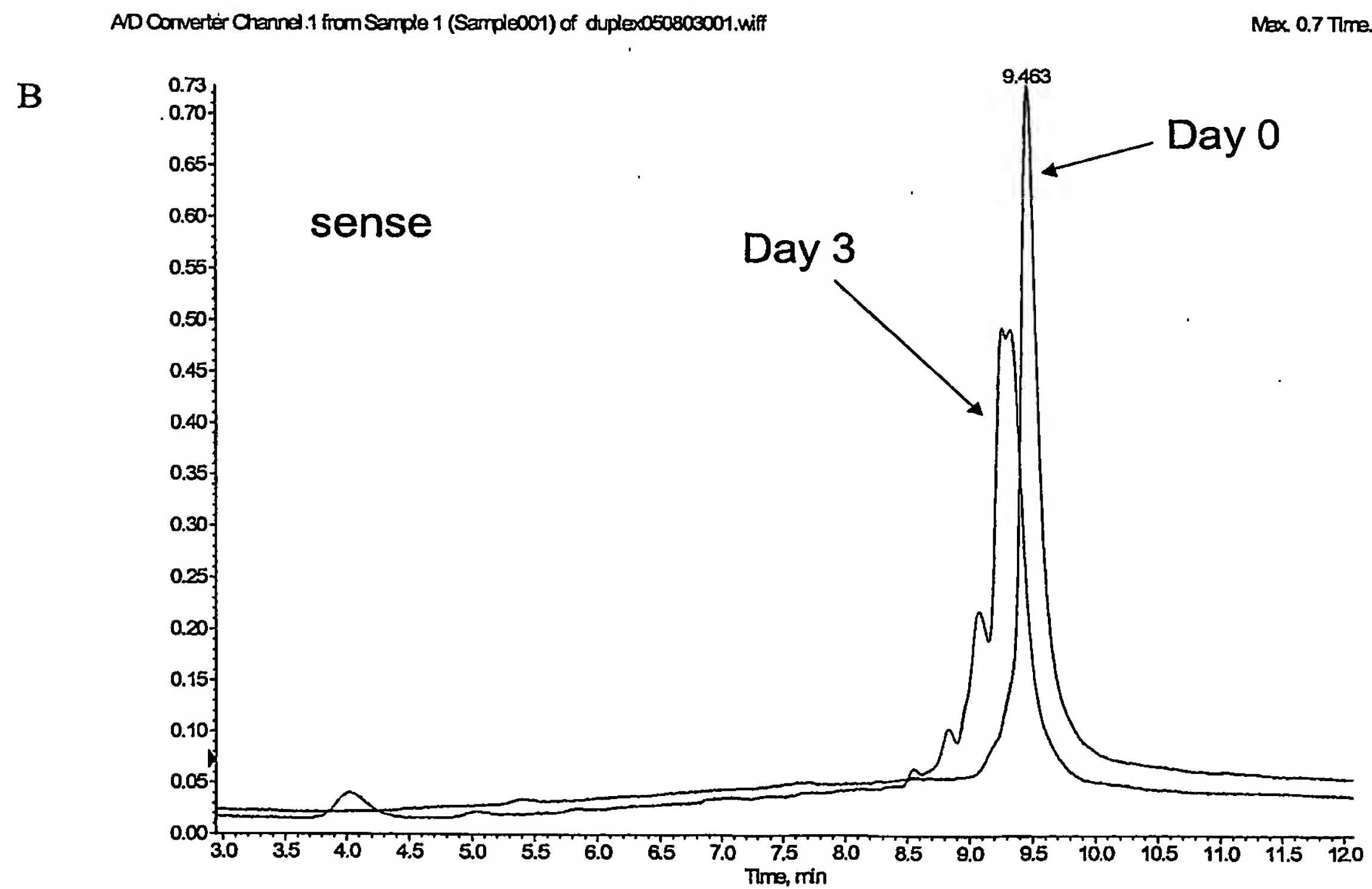
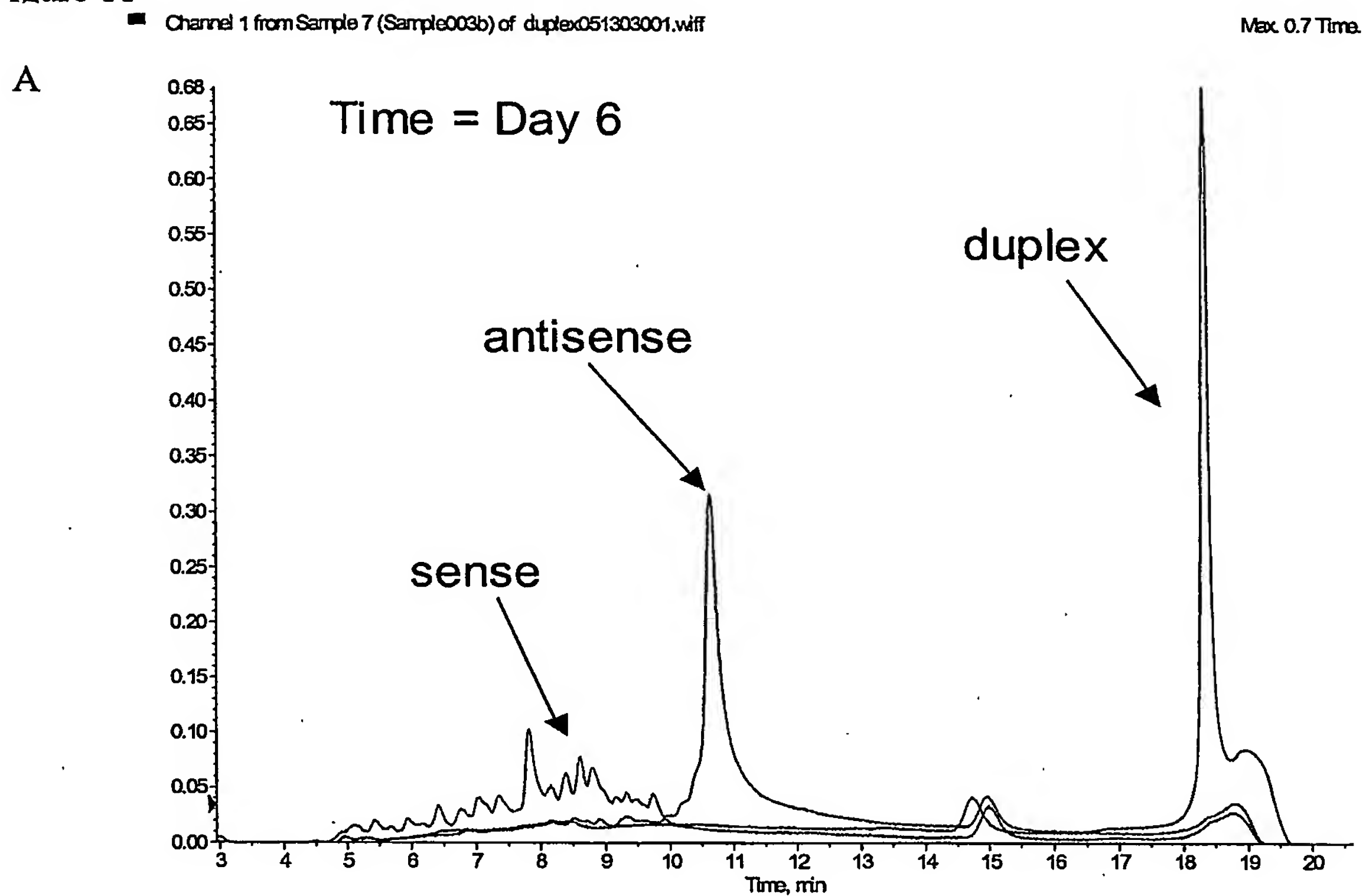
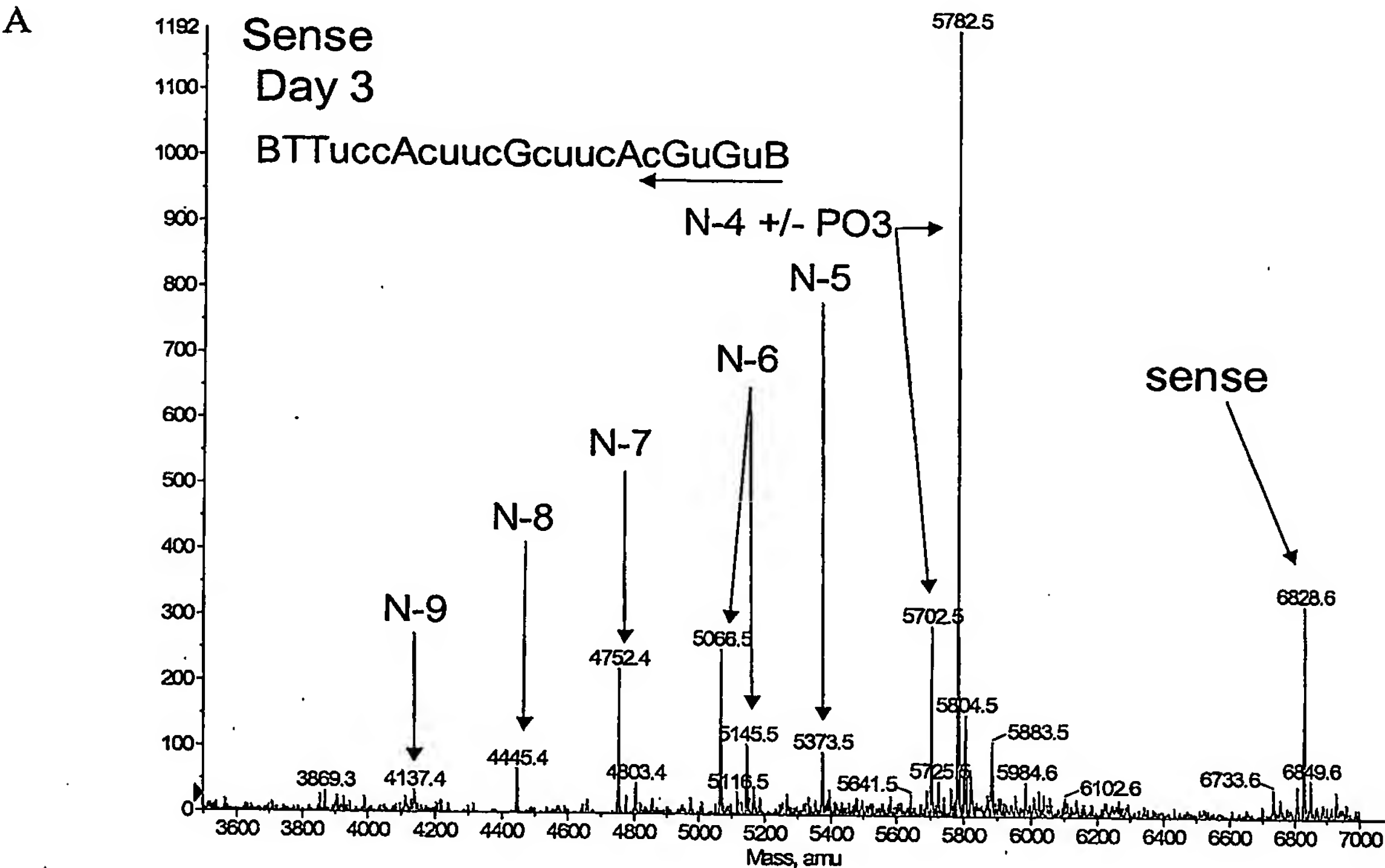


Figure 12

... instruction of -Q1: 8.643 to 11.023 min from Sample 1 (Sample001) of duplex050903002.wiff Max. 1.0 cps.



Mass reconstruction of -Q1: 9.520 to 10.981 min from Sample 2 (Sample002) of duplex051603001.wiff Max. 1.0 cps.

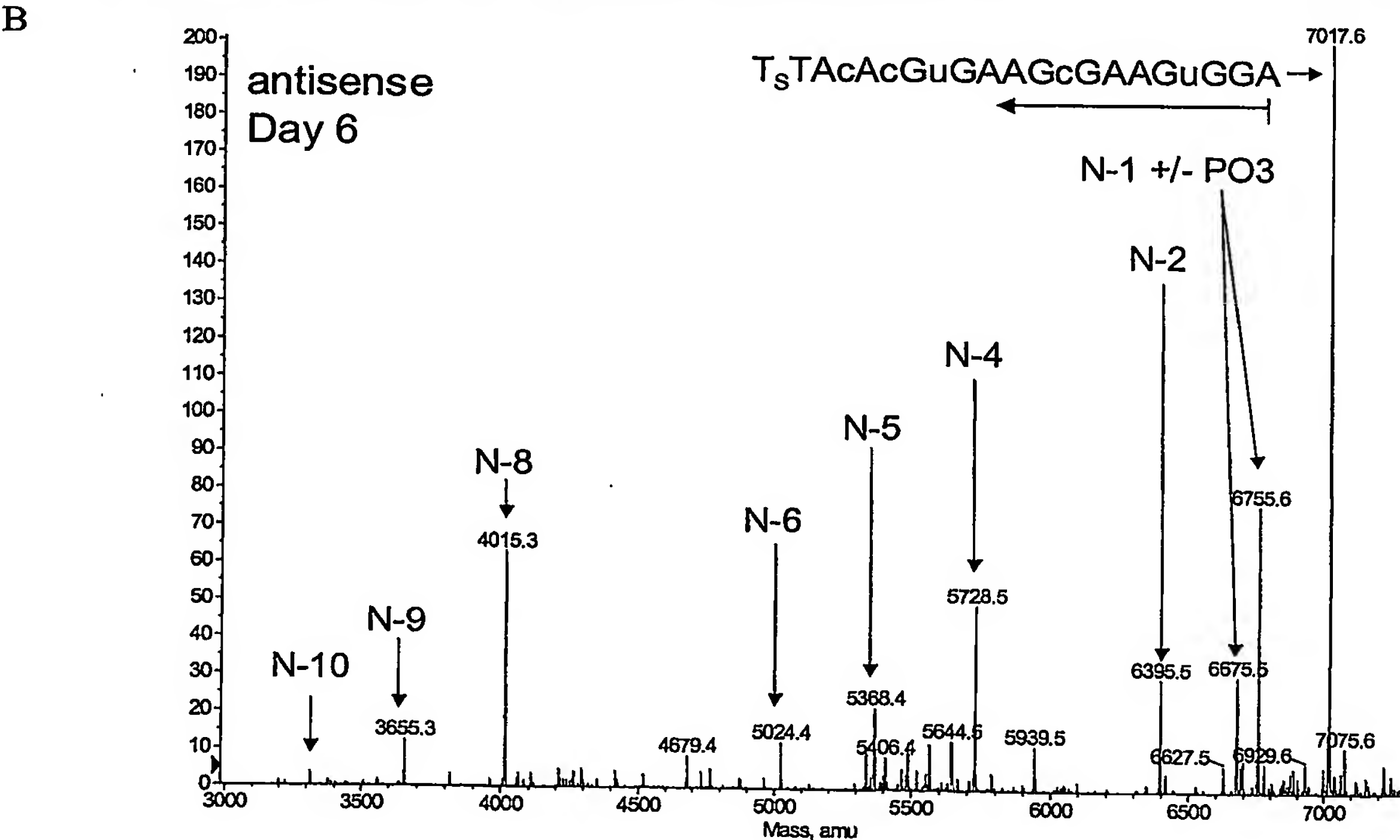


Figure 13

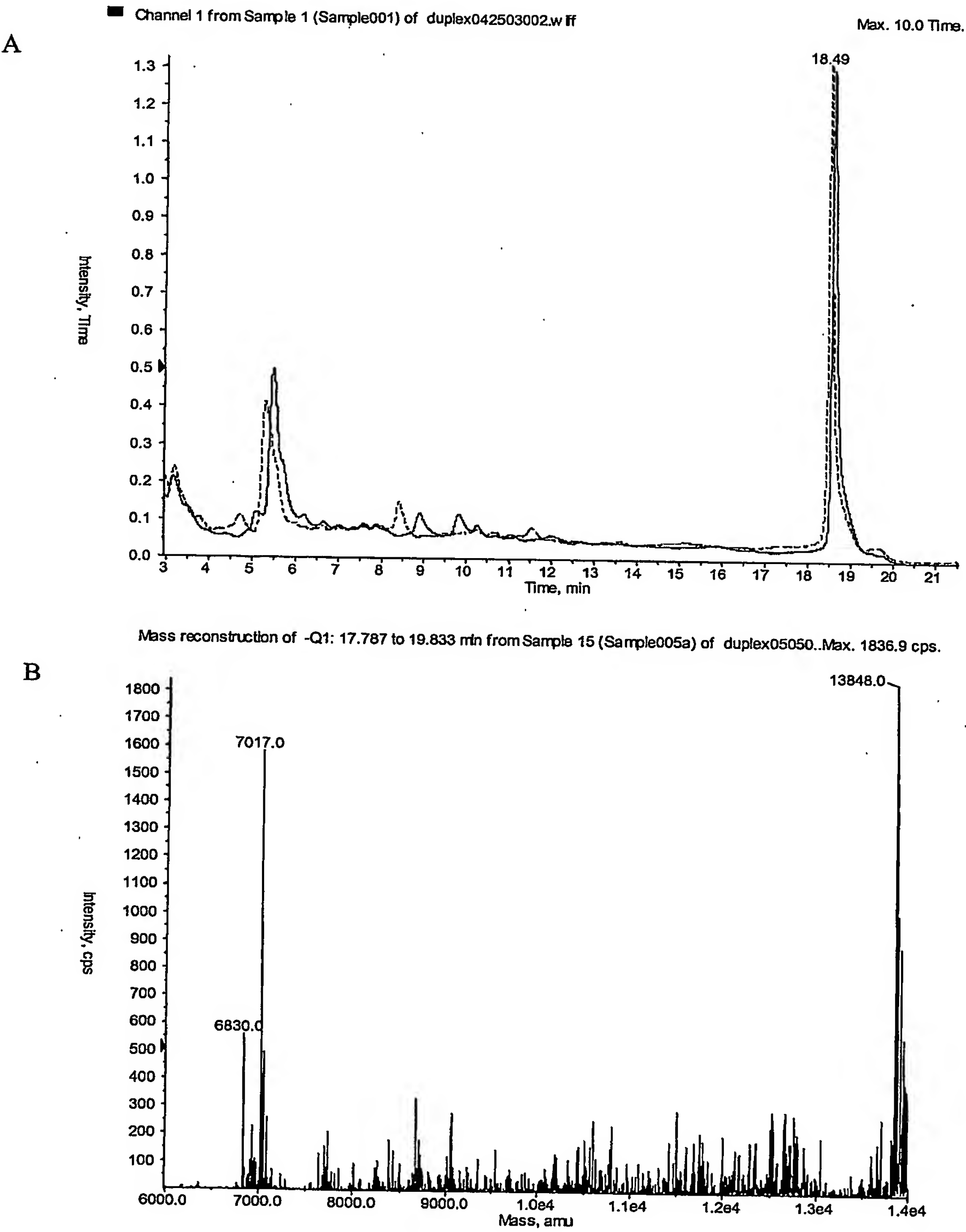


Figure 14

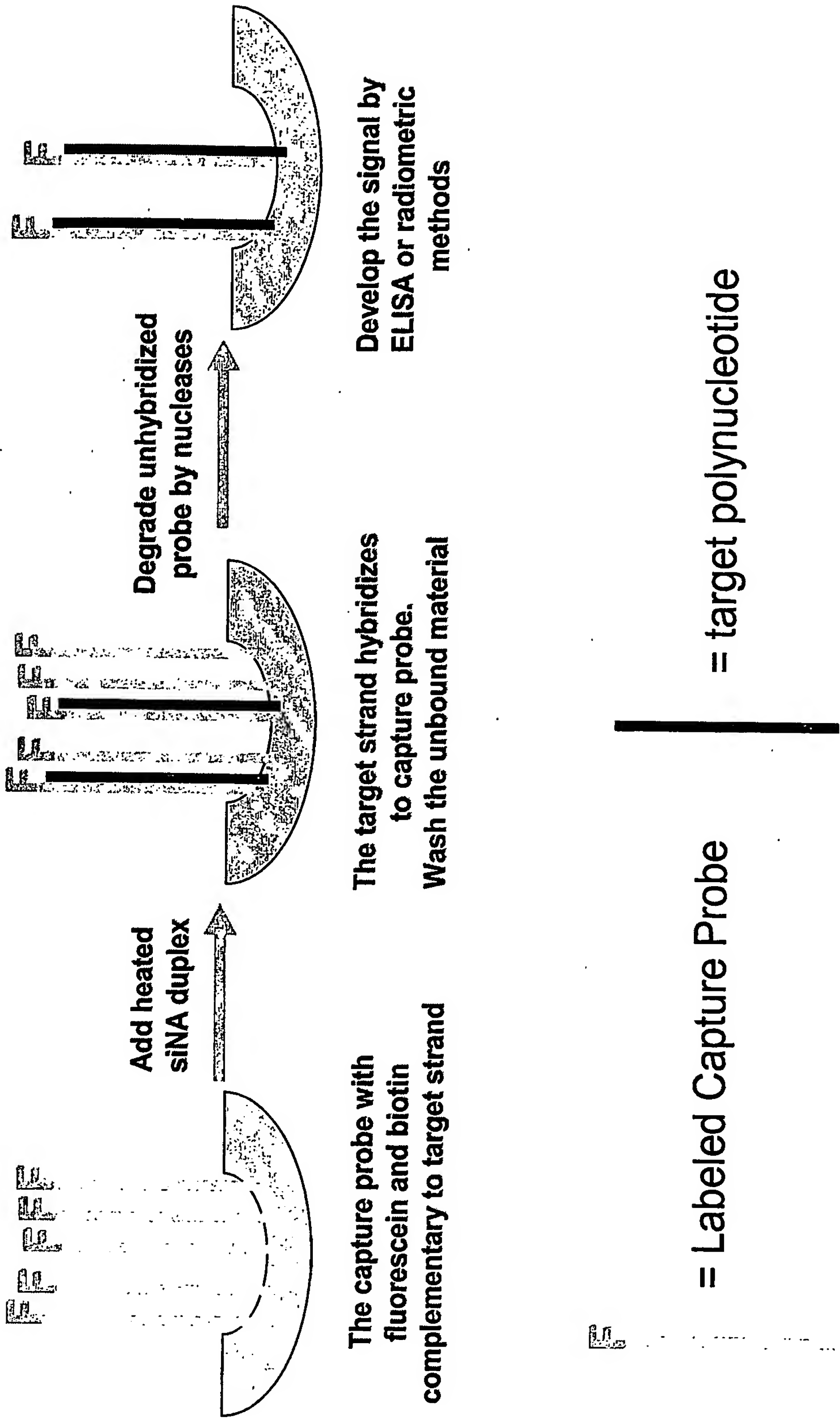
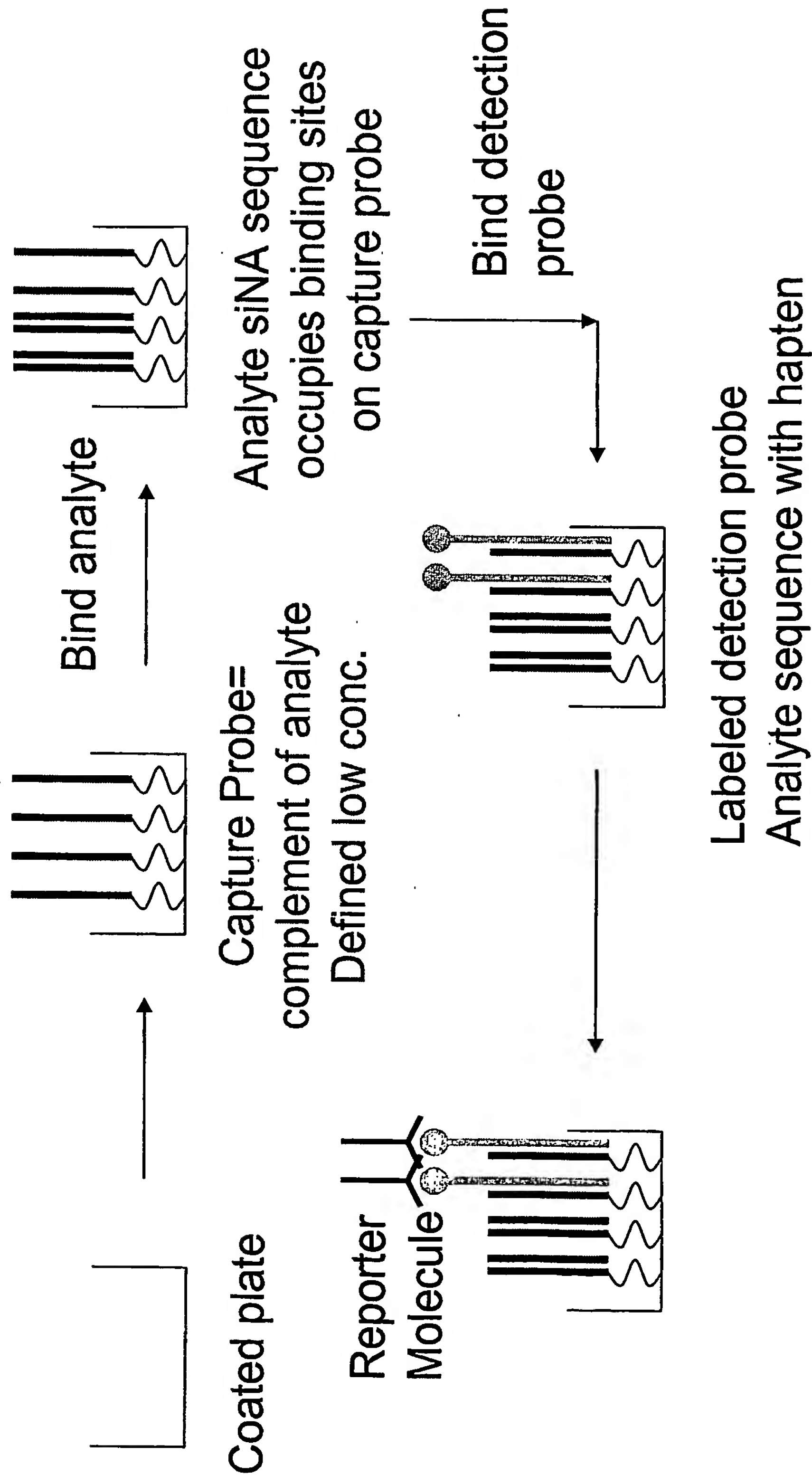


Figure 15



In this design, binding of the target siRNA (in step 1) prevents binding of a secondary detection probe (in step 2). Therefore, signal is inversely proportional to analyte concentration.